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Drosophila long-chain acyl-CoA synthetase acts like a gap gene in embryonic segmentation

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ABSTRACT

Long-chain acyl-CoA synthetases (ACSLs) convert the long chain fatty acids to acyl-CoA esters, the activated forms participating in diverse metabolic and signaling pathways. dAcsl is the *Drosophila* homolog of human ACSL4 and their functions are highly conserved in the processes ranging from lipid metabolism to the establishment of visual wiring. In this study, we demonstrate that both maternal and zygotic *dAcsl* are required for embryonic segmentation. The abdominal segmentation defects of *dAcsl* mutants resemble those of gap gene *knirps (kni)*. The central expression domain of Kni transcripts or proteins was reduced whereas the adjacent domains of another gap gene Hunchback (Hb) were correspondingly expanded in these mutants. Consequently, the striped pattern of the pair-rule gene Even-skipped (Eve) was disrupted. We propose that *dAcsl* plays a role in embryonic segmentation at least by shifting the anteroposterior boundaries of two gap genes.

Introduction

In *Drosophila* embryo, a hierarchy of maternal, gap, pair-rule and segment polarity genes which encode transcription factors establish the anteroposterior axis and the embryonic segmentation (St Johnston and Nusslein-Volhard, 1992). The spatially restricted transcription factors determine the complex gene expression patterns in the early embryo. Along with the maternal determinants, the gap gene products specify the boundaries of the adjacent gap gene expression domains and the downstream pair-rule gene stripes (Hulskamp et al., 1990; Pankratz et al., 1990; Struhl et al., 1992). Among them, Knirps (Kni) and Hunchback (Hb) form their expression patterns partly through mutual repression (Clyde et al., 2003; Yu and Small, 2008).

The known maternal effectors are not sufficient to establish the gap domains and it is likely that unidentified maternal molecules exist and modulate the gap gene expression (Jaeger et al., 2007). The abundant maternally-deposited lipids in embryos have been recognized as an energy source for early embryo development. These molecules also have important functions in diverse signaling pathways during larval development such as shaping morphogen gradients (Eaton, 2008; Hausmann et al., 2007). However, it remains unclear whether lipids participate in any way in the establishment of embryonic segmentation.

Long chain acyl-CoA synthetase (ACSL) is a family of enzymes which adds Coenzyme A to the long chain (C12-20) fatty acids (Soupene and Kuypers, 2008). As the activated form of fatty acids, the Acyl-CoA participates in various cellular processes including lipid metabolism, vesicle trafficking and signal transduction. ACSL4 is a member of the mammalian ACSL family and its mutations have been associated with non-syndromic X-linked mental retardation (MRX) (Longo et al., 2003; Meloni et al., 2002; Piccini et al., 1998). The *Drosophila* gene *dAcsl* encodes the homolog of human ACSL4 and they are functionally conserved ranging from building visual circuitry to lipid homeostasis (Zhang et al., 2009). However, the developmental function of *dAcsl* at the embryonic stages remains unexplored.

In this report, we illustrate that *dAcsl* is required for embryonic segmentation both maternally and zygotically. The impaired segmentation caused by *dAcsl* mutations is similar to that of gap gene *kni* mutants. In *dAcsl* mutants, the domain of Kni transcripts or proteins was reduced whereas the domain of another gap gene Hb protein was correspondingly expanded. Consequently, the pair-rule gene expressions were perturbed in these embryos. We propose that *dAcsl* participates in embryonic segmentation by spatially modulating gap gene expression.

Materials and methods

Genetics and stocks

dAcsl^{1}, *dAcsl*^{8}, *Df(2R)H3E1*, *knirps*^{1}, *Kruppel*^{1}, *hunchback*^{12}, and *Lsd2*^{KG00149} (*Lsd2*^{KG}) were obtained from Bloomington Stock Center; *PBac(RB)dAcsl*^{e02676} and *PBac(WH)dAcsl*^{f02764} from Harvard collection; *Lsd2*^{NP0141} from Kyoto *Drosophila* Genetic Resource Centre. *dAcsl*^{KO} was generated by precisely deleting the DNA sequence between *PBac(R)*

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dAcsl^{e02676} and *PBac(WH)dAcsl*^{f02764}, removing nearly all *dAcsl* coding sequence except for its first 29 nucleotides. All crosses were done at 25 °C unless specified. Molecular information of allele *dAcsl*^{1} and *dAcsl*^{8} has been described (Zhang et al., 2009). To get the maternal *dAcsl* mutants, we generated the homozygous germ line clones using the dominant female sterile technique (Chou et al., 1993; Chou and Perrimon, 1996). The pupae were heat shocked in 37 °C incubator for an hour and twice each day with an interval of ~10 hours. The virgin females of *hsFLP; FRT 42B ovo*^{D}*/FRT 42B dAcsl*^{1} and *hsFLP; FRT 42B ovo*^{D}*/FRT 42B dAcsl*^{8} were crossed to *dAcsl*^{1}*/CyO Kr>GFP, dAcsl*^{8}*/CyO Kr>GFP*, or *dAcsl*^{KO}*/CyO Kr>GFP*. The embryos were collected and allowed to develop until the 1st instar (L1) larvae. The maternal and zygotic mutants were distinguished by GFP marker. The *dAcsl M*$^{-}$ embryos were the progenies of heat-shocked females of *hsFLP; FRT42B ovo*^{D}*/FRT42B dAcsl*^{1} mated to *dAcsl*^{KO}*/CyO GFP*. These embryos included two zygotic genotypes which were undistinguishable before Stage 7.

In situ hybridization

Digoxigenin-labeled sense and anti-sense RNA probes from dAcsl cDNA clone (LP07340) or clones containing PCR-amplified *kni* cDNA sequence. Whole-mount in situ hybridizations to embryos were performed as described (Tautz and Pfeifle, 1989). The stained embryos were mounted in 70% glycerol and photographed using Zeiss AxioPlan2 microscopic system (DIC objectives and AxioCam MRm or ProgRes C5 cool camera).

Immunostaining

All samples were fixed and stained according to the standard embryo antibody staining protocol (Patel, 1994). Primary antibodies were used at the following dilutions: guinea pig anti-Hb at 1:300, guinea pig anti-Kni at 1:300, guinea pig anti-Kruppel (Kr) at 1:300 (these are from Asian Distribution Center for Segmentation Antibodies, National Institute of Genetics, Japan) (Kosman et al., 1998); Rabbit anti-Bcd at 1:500 (from Gary Stuhl); Rabbit anti-Nos at 1:2000 (from Dahua Chen); mouse anti-Eve (2B8, DSHB) at 1:100. Guinea pig anti-dAcsl antibody was used at a 1:1000 dilution for immunostaining and the specificity of this antibody has been described previously (Zhang et al., 2009). AlexaFluor conjugated secondary antibodies (Molecular Probes of Invitrogen) were used at 1:4000. Fluorescent images were collected by Zeiss ApoTome or Olympus FV1000 Confocal microimaging system.

Cuticle preparation

The embryos of specific genotypes were collected and allowed to develop until the L1 larvae, then placed in the Hoyer's medium and heated at 60 °C for more than 2 hours or until they became clear. The embryos with defective segmentation referred to those having one or more segments loss, or partial loss with segment fusions.

Quantitative analysis of gap gene stripes

The Kni and Hb domains referred to those having visible Kni and Hb nuclear staining. They were outlined and measured using the NIH Image J program and normalized against the total image area of each embryo. For all experiments, *p* values are the results of Student's *t*-test provided by Microsoft Excel (*$p<0.05$; **$p<0.01$). The error bar represents the standard error of mean.

Results

dAcsl is maternally deposited and ubiquitously expressed in the early embryos

Our previous work has shown that *dAcsl* is ubiquitously expressed in the larval tissues and enriched in the ER (Zhang et al., 2009). To evaluate how dAcsl is expressed in the embryonic stages, we examined its expression at mRNA and protein levels. By in situ hybridization, we found that *dAcsl* mRNA is maternally deposited (Fig. 1A and B). Using a

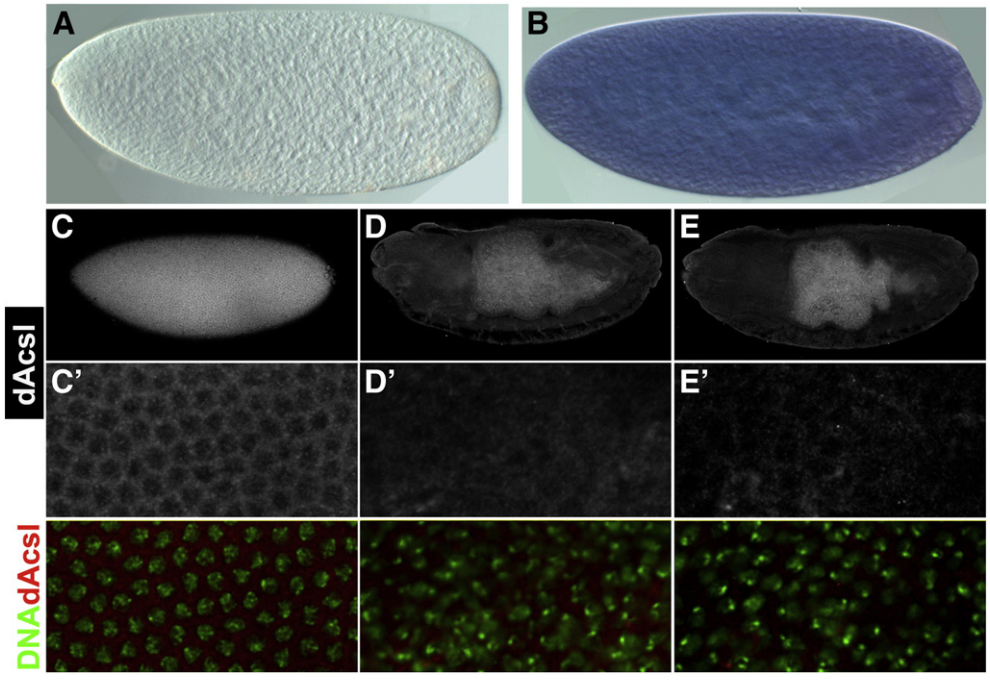

Fig. 1. *dAcsl* is maternally deposited and ubiquitously expressed in early embryos.(A and B) Syncytial blastoderm embryos hybridized with sense (A) or anti-sense (B) *dAcsl* RNA probes. Since the anti-sense probe was generated using the identical region of all *dAcsl* isoforms, it did not distinguish the expression of different isoforms. (C to E') The ubiquitous expression of dAcsl detected by anti-dAcsl antibody, whose specificity in embryo is shown by the strong staining in the wild type cellularized embryo (C and C'), weak staining in the wild type Stage 16 embryo (D and D'), and poor staining in the Stage 16 embryo of *dAcsl*^{KO} (E and E'). Images in C–E or in C'–E' were taken at the same confocal settings. Images in C'–E' are the higher magnifications of the embryos in C–E, respectively. The focal planes for the sections in C'–E' were revealed by the DNA co-staining in the bottom panels.